

APPENDIX A
MARKINGS TO SHOW CHANGES MADE

The paragraph at page 8, beginning at line 28, has been amended as follows:

Figure 11. DNA (SEQ ID NO: 7) and peptide (SEQ ID NO: 8) sequence of W985.

The paragraph at page 9, beginning at line 22, has been amended as follows:

Sequence of oligo primers used to amplify RV-14 cDNA fragments (rh1A.F1 (SEQ ID NO:9); rh1A.R1 (SEQ ID NO: 10); rh1B.F1 (SEQ ID NO: 11); rh1B.R1 (SEQ ID NO: 12); rh1C.F1 (SEQ ID NO: 13); rh1C.R1 (SEQ ID NO: 14); rh1D.F1 (SEQ ID NO: 15); rh1D.R2 (SEQ ID NO: 16); rh2A.F1 (SEQ ID NO: 17); rh2A.R1 (SEQ ID NO: 18); rh2B.F1 (SEQ ID NO: 19); rh2B.R2 (SEQ ID NO: 20); rh2C.F1 (SEQ ID NO: 21); rh2C.R1 (SEQ ID NO: 22); rh3A/B.F1 (SEQ ID NO: 23); rh3A/B.R1 (SEQ ID NO: 24); rh3C.F1 (SEQ ID NO: 25); rh3C.R2 (SEQ ID NO: 26); rh3D.F1 (SEQ ID NO: 27); rh3D.R1 (SEQ ID NO: 28)).

The paragraph at page 76, beginning at line 7, has been amended as follows:

Using techniques that are familiar to individuals in the art, randomly primed cDNA libraries were used as a source of sequences encoding putative anti-Rhinovirus antiviral agents. As one non-limiting example of how to construct such a library, polyA mRNA derived from placental tissue was PCR amplified using a random 9-mer linked to a unique SfiI sequence ("SfiA"), followed by an additional sequence that is used later for library amplification (OVT 906: 5' ACTCTGGACTAGGCAGGTTCAGTGGCCATTATGGCC(N)₉(SEQ ID NO: 1). The product of this reaction was size selected (>400 base pairs) and subjected to RNase A/H treatment to remove the original RNA template. The remaining single stranded DNA was then subjected to a second round of PCR using a random hexamer nucleotide sequence linked to a second unique SfiI sequence ("SfiB") which was again followed by an additional sequence for future library amplification: (OVT 908: 5' AAGCAGTGGTGTCAACG CAGTGAGGCCGAGGCCGCC (N)₆(SEQ ID NO: 2). The final product of this reaction, a double stranded cDNA, was blunted/filled with Klenow Fragment (New England BioLabs), size selected, PCR amplified (OVT 909: 5' ACTCTGGACTAGGCAGGTTCAAGT (SEQ ID NO: 3)

and OVT 910: 5' AAGCAGTGGTGTCAA CGCAGTGA (SEQ ID NO: 4)), digested with SfiI (New England BioLabs), and inserted into a retroviral vector (pVT 352.1, pBabe). As a result of these procedures, the sequences encoding the perturbagens were inserted at the 3' end of the non-fluorescent variant of EGFP (dead GFP or "dEGFP"). Expression of the dEGFP-perturbagen fusion gene (as well as the neomycin resistance gene present in the retroviral vector) was driven by the 5' LTR of pBabe. The library (~12 x 10⁶ in size) was then packaged in 293gp cells (laboratory of I. Verma) and retroviral supernatant was collected over the course of the following 48-72 hours. Two methods are commonly used for retroviral packaging. In the first technique, the retroviral library is co-transfected with VSV-G envelope expression plasmid into 293gp packaging cells (gift of I. Verma, Salk Institute) using LIPOFECTAMINE (Life Technologies). In this technique, 3 x 10⁶ cells of the packaging cell line (293gp) are seeded into a T175 flask. On the next day, two tubes are prepared, one containing 15 g of library DNA and 10 g of envelope plasmid (pCMV-VSV.G-bpa) in 1.5 ml DMEM (serum free), the second containing 100 g of LIPOFECTAMINE in 1.5 ml DMEM (serum free). These tubes are incubated at room temperature for 30 minutes, mixed and incubated for another 30 minutes. Subsequently the mix is added to 17 mls of serum free DMEM. This mix was added to previously plated 293gp cells which had been washed with serum free media. Following a 4 hour incubation at 37°C. The transfection mix was removed and the cells are washed once in DMEM containing 10% serum and left in the same media. After 72 hours at 37°C the media (now referred to as "viral supernatant") is collected, filtered through a 0.45μm filter and frozen at -80°C. It is possible to make a second collection of virus which has a comparable titer by adding 15mls of DMEM (10% serum) back to the cells and incubating a further 24 hours.

The paragraph at page 80, beginning at line 12, has been amended as follows:

Following each cycle, live, adherent cells were collected and used to prepare a new sublibrary. The procedure of retrieving the library sequences after each successive round of selection minimizes the background levels of viral-resistant cells that can accumulate due to mutations in the host chromosomal DNA. As one example of generating a perturbagen sublibrary, adherent cells that had been harvested by trypsinization of the culture flask were collected by centrifugation and used to prepare genomic DNA (Trizol, Reagent, Life Technologies). The library DNA was then recovered by two stages of PCR amplification using oligonucleotides that contained homology with sequences flanking the cDNA insertion site

(oVT181: 5' GGATCACTCTGGCATGGACGAG (SEQ ID NO: 5) and oVT178: 5' ATTTATCGATGTTAGCTTGGCCATT (SEQ ID NO: 6)). Specifically genomic DNA from 10,000 to 700,000 cells was added to a 100 ul PCR containing 2.5mM MgSO₄, 10 μ M primers, 0.2 mM dNTPs, 100ug/ml BSA and 10 units HiFi Taq polymerase (Life Technologies) in 1x buffer supplied by the manufacturer. This was denatured at 94°C for 5 minutes and then amplified by 20 cycles of: 94°C 15 seconds, 68 C for 2:20 minutes followed by 5' at 68C. Ten microliters of this reaction was further amplified in a 200 ul PCR reaction under the same conditions for a number of cycles determined by cycle course titration (generally 16 cycles).